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FORMULATION AND EVALUATION OF COMPRESSION COATED TABLETS OF MESALAZINE FOR COLON DELIVERY

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Abstract: Colon specific drug delivery system based on a natural polysaccharide; locust bean gum (550, 450, 350 & 250mg) was evaluated by *in vitro* and *in vivo* methods. The *in vitro* studies in pH-6.8 phosphate buffer containing 4% w/v of rat caecal contents showed the cumulative percentage release of Mesalazine after 26h as 33.75% ±0.1988, 46.25 % ± 0.9640 and 95.75 % ± 0.1013 respectively. These studies on the polysaccharide indicated that locust bean gum as a coating material, proved capable of protecting the core tablet containing Mesalazne under conditions mimicking mouth to colon transit. The *in vivo* studies conducted in nine healthy human volunteers for the various formulations revealed that the drug release was initiated only after 5 h (i.e.) transit time of stomach, small intestine and the Bioavailability (AUC_{0-t}) of the drug was found to be 147.985±0.5, 249.57±0.17, 480.075±0.069µg h\ ml respectively. This study clearly established that locust bean gum in the form of compression coat is a potential carrier for drug targeting to the colon.

Key words: Colon targeting; Polysaccharide; Locust bean gum; Mesalazine

1. Introduction

Drug targeting to colon is highly desirable in a variety of colonic disorder such as inflammatory bowl disease (IBD), infectious disease and colon cancer. There are three practical mechanisms for targeting orally administered drug to colon, in which the use of bacterially triggered delivery system is considered to be best when compared to the pH dependent coating and time dependent coating. In this work, we have used locust bean gum as a polysaccharide to coat the Mesalazine core tablets, which are degraded by colonic bacteria to deliver the drug and their efficacy in colon targeting, was studied. The different polysaccharides under evaluation as carriers for colonic drug delivery include pectin and its salts (Ashford et al., 1993b, 1994; Rubinstein et al., 1993; Wakerly et al., 1996a, b) chondroitin sulphate (Rubinstein et al., 1992a,b) amylose (Milojevic et al., 1995) inulinHP (Vervoort an Kinget, 1996). In another study guar gum was used as a compression coat polymer that delayed the drug release in the small

intestine by forming swellable layer around the drug core and is degraded by colonic bacterial enzymes, thereby releasing the drug in the colon (Rama prasad, Y.V.et al., 1998, Krishnaiah Y.S.R. et al., 1998). This formed the basis of investigating the usefulness of locust bean gum (containing polygalactomannans like guar gum) as a carrier for drug targeting to the colon. Further, Mesalazine, 5-amino salicylic acid is very effective in the treatment of inflammatory bowl diseases. However systemic circulation of the drug may be associated with acute pancreatitis (Issacs K.C. & Murthy D. 1990) and nephrotoxicity (Navis B.H. et al., 1998). This may be optimized with a control drug delivery system, which maximizes topical exposure of the drug to the diseased tissue and minimizes systemic absorption of the drug. Locust bean gum is a high molecular weight (3,10,000) hydro colloidal polysaccharide derived from the endosperm of the seed of *Ceratonia siliqua Linn* (Family- Leguminosae). The physical properties of Locust bean gum are similar to those of guaran and the two gums can be used interchangeably in pharmaceutical preparations. The gum contains 88% D-galacto-D-mannoglycan, 4% pentan, 6% of proteins, 1% cellulose and 1% ash. The structure of Locust bean gum differs from the structure of guaran only in the smaller number of D-galactosyl units as side chain. In pharmaceutical formulations, Locust bean gum is used as a binder, flocculating agent, thickening and stabilizing agent. In the present investigation, locust bean gum in the form of compression coat applied over core tablets was evaluated as a suitable carrier for colonic drug delivery. *In vitro* drug release studies were carried out on Mesalazine core tablets compression coated with different quantities of locust bean gum in simulated gastro intestinal fluids in the presence and absence of rat caecal contents.

2. Materials and methods

2.1. Materials:

Locust bean gum was obtained from Fluka Biochemica, Switzerland. Mesalazine (5-amino salicylic acid) was obtained from Sun Pharmaceuticals Ltd., India. Microcrystalline cellulose and magnesium stearate were obtained from Loba chemic Pvt. Ltd., India. Starch was obtained from E-Merck (India) Pvt. Ltd., India. Talc and Sodium lauryl sulphate were obtained from S.D.Fine chem. Ltd., India.

2.2. Preparation of Core tablets:

Each core tablet (average weight 80mg) for *in vitro* and *in vivo* drug release studies consists of Mesalazine (40mg), microcrystalline cellulose (MCC, 29mg), dried starch (5mg), sodium lauryl sulphate (4 mg), talc (1.5mg) and magnesium stearate (0.5mg). Starch and sodium lauryl sulphate were added to obtain fast disintegration tablets (disintegration time <1min) of Mesalazine. The materials were weighed, mixed and passed through a mesh (250µm) to ensure complete mixing. The tablets were prepared by compressing the thoroughly mixed materials using 6 mm round, flat and plain punches on a single station tablet machine (Cadmach India). The thickness of the core tablet was 2mm and their crushing strength was checked at $3kg/cm^2$.

2.3. Preparation of Compression coated tablets:

The formulated core tablets were compression-coated with different quantities of coating material containing 550, 450, 350 and 250 mg of Locust bean gum (Table 1). Since Locust bean gum alone gave very soft coats, microcrystalline cellulose was included in the coat formulations to impart enough hardness. The compression coating was provided by placing half the quantity of coating material in the die cavity, then the core tablet was carefully positioned in the center and filled with the other half of the coating material. The coating material was compressed at the pressure of 5000kg using 9mm round, flat and plain punches. The crushing strength of the tablet was $5kg/cm^2$.

2.4. In vitro drug release studies:

The compression coated Mesalazine tablets were evaluated for their integrity in the physiological environment of stomach and small intestine under conditions mimicking mouth to colon transit. These studies were carried out using a USP XXII / XXIII dissolution rate test apparatus (Apparatus 1, 100rpm, 37 ºC). The tablets were tested for the drug release for 2 h in 0.1N HCl (900 ml), as the average gastric emptying time is about 2 h. Then the dissolution medium was replaced with pH 7.4 phosphate buffer (900 ml) and tested for 3 h, as the average small intestine transit time is about 3 h. At the end of the time periods, two samples each of 1ml were taken, suitably diluted and analysed for Mesalazine content using spectrofluorimeter (Jasco FP-750).

The susceptibility of locust bean gum coats to the enzymatic action of colonic bacteria was assessed by continuing the drug release studies in 100 ml of pH 6.8 phosphate buffered saline (PBS) containing 4%w/v of rat caecal contents. The caecal contents were obtained from male albino rats (obtained from Pasteur institute, Coonoor, Nilgiris, Tamil Nadu, India) after pretreatment for 7 days with locust bean gum dispersion, as the studies earlier have shown the presence of 4% w/v rat caecal contents in pH 6.8 PBS obtained after 7 days pre-treatment of rats with 1 ml of 2% w/v of locust bean gum have provided the best conditions for *in vitro* evaluation on Locust bean gum (Rama Prasad et.al., 1998). Thirty minutes before the studies, the rats were killed by spinal traction. The abdomen were opened, the caecai were isolated, ligated at both ends, dissected and immediately transferred into pH 6.8 PBS previously bubbled with

CO_2. The caecal bags were opened; their contents were individually weighed, pooled and then suspended in PBS to give a final caecal dilution of 4% w/v. As the caecum is naturally anaerobic, all these operations were carried out under CO_2. The drug release studies were carried out in USP dissolution rate test apparatus (apparatus 1, 100rpm, 37^0C) with slight modification (Krishaniah Y. S. R. et al., 1998). A beaker (capacity 150 ml, internal diameter 55 mm) containing 100 ml of dissolution medium was immersed in 1000 ml vessel containing water, which was in turn in the water bath of the apparatus. The coated tablets were placed in the baskets of the apparatus and immersed in the dissolution medium containing rat caecal contents. The experiments were carried out with continuous CO_2 supply into the beakers to simulate anaerobic environment of the caecum. The drug release studies were carried out for 21 h (usual colonic transit time is 20-30 h) and 1 ml samples were taken at different time intervals and replaced with 1 ml of fresh PBS bubbled with CO_2. The volume was made upto 10 ml with PBS, centrifuged and the supernatant liquid was filtered through bacteria proof filter and analysed for Mesalazine spectro-fluorimetrically (Ramuseen S. et al., 1982). The above study was carried out for all the Mesalazine coated tablets without rat caecal content in pH-6.8 PBS (control).

2.5. In vivo Drug studies:

For *in vivo* studies of the usefulness of Locust bean gum in the colon drug delivery, nine male healthy human volunteers of 20-23 years age and 55-70 kg weight were selected. They were non-alcoholics, non-smokers and were not on any drugs. The purpose of the study was fully explained and each volunteer had given their written consent and had been approved by the ethical committee of the institution. Their liver and kidney function were assessed to be normal by clinical and standard biochemical investigation. A 9 x 3 complete cross over design was carried out in nine healthy male volunteers. After overnight fasting, the three different coated formulations F1, F2, F3 (Table 1) of Mesalazine were given to the volunteers along with 200ml of water. The food was with held for a period of 2 h. The blood samples were collected at 0, 3, 5, 7, 10, 13, 16,19, 22, 25, 28 & 31h. The plasma was separated, drug was extracted (Atterman K, et al., 1980) and analysed by spectro-fluorimetry (Rasmuseen S. N. et al., 1982).

2.6. Data Analysis:

Data were generated by assuming the first order absorption and one compartment model with first order elimination. The maximum peak concentration (Cmax) and time of its occurance (Tmax) were directly computed from the plasma concentration versus time plot. The elimination rate constant (Kel) was determined from the terminal phase of the log plasma concentration versus time profile by least square regression analysis. From this Kel was calculated as Kel = slope x 2.303. The elimination half-life was calculated as $t_{1/2}$ = 0.693/Kel. The area under the plasma concentration time curve from 0→t* ($AUC_{0\rightarrow t^*}$) and from 0-∞ ($AUC_{0-\infty}$) and mean residence time (MRT) was calculated using trapezoidal rule.

3. Results

3.1. In vitro Drug release studies:

The percentage of drug release at different time periods from the Mesalazine tablet compression coated with coat formulation F1, F2 and F3 in 0.1N HCl (2 h), pH 7.4 phosphate buffer (3 h) and pH 6.8 phosphate buffer saline (21 h) are shown in Fig. 1. The result of the drug release studies carried out in the presence of 4% w/v rat caecal contents in pH 6.8 PBS are shown in Fig. 2. From the above-mentioned figures it was clear that the drug was not released till 5 h that indicated that the drug was not released in the presence of 0.1N HCl and pH 7.4 - phosphate buffer.

3.2. In vivo Drug release studies:

In vivo studies conducted in nine healthy human volunteer for the various formulation revealed that, the drug release was initiated only after 5 h.The blood samples collected from the healthy male volunteers showed different pharmacokinetic parameters for the different formulations. The parameters were shown in Table 2, and the mean plasma concentrations of different formulations are given in Fig 3. The pharmacokinetic parameters and the mean plasma concentration showed that the drug from all the formulation was released only.

4. Discussion

The successful delivery of drugs to the colon requires the protection of drug from being released in stomach and small intestine. The present investigation, locust bean gum in the form of compression coat was applied over Mesalazine core tablet and drug release studies were carried out condition mimicking mouth to colon transit. The *in vitro* release of the drug from tablets coated with coat formulation containing 600, 500 & 400 mg of locust bean gum (F1, F2, F3) was not found till 5 h of testing in simulated gastric and intestinal fluid (Fig.1). But on exposure to the dissolution fluid, the gum got hydrated and formed a viscous gel layer that slowed down further sleeping-in of dissolution fluid towards the core tablets. The hydration of locust bean gum seemed not to be affected by pH of the dissolution medium. Thus, locust bean gum in the form of coat was capable of protecting the drug from being released completely in the physiological environment of stomach and small intestine. However the tablet with coat formulation F4 was found disintegrated within 30 min in pH 7.4 - phosphate

buffer and this may be due to lesser gum content (300mg) of the coat, which was unable to remain intact, and failed to protect the drug core from being released. To assess the integrity of the coats, the drug release was further continued for 21 h by replacing the dissolution medium with pH 6.8 PBS. At the end of experiment (26 h), the cumulative mean percentage (S.D) of drug released from the coat formulations F1,F2 & F3 were found to be 33.75% ± 0.1988, 46.25 % ± 0.9640, 95.75 % ± 0.1013 respectively. This indicated that gum coat would not permit the release of the bulk of the drug until the coat was broken. The aim of drug delivery system targeted to the colon is not only to protect the drug from being released in the physiological environment of stomach and intestine, but also to release the drug in the colon after enzymatic degradation of colonic bacteria. Hence, the *in vitro* drug release studies were carried out in pH 6.8 PBS containing 4% w/v of rat caecal contents. At the end of 26 h of testing which included testing in simulated gastric and intestinal fluid, the percent of Mesalazine released from the coated tablets with formulation F1 was found to be only 33.75 % 0.1988, and there was no rapid increase in the delivery of drug to the colon. The formulation F2 was found to be 46.25 % ± 0.9640, and there was small increase in the release of drug after 14 h. The formulation F3 showed a rapid increase of drug release after 13 h and the cumulative mean percentage release of the drug at 26 h was 95.75% ± 0.1013. The release rate showed that the coat formulation F3 (400mg of locust bean gum) produced better release of Mesalazine. About 95.75% of the drug was released in the colon after protecting drug from the stomach and small intestine. It was also evident from the results of drug release the presence and absence of rat caecal contents that the maximum amount of drug release occurred by the degradation of the coat material by the enzymes present in the caecal content. Even though the *in vitro* studies had revealed that the better release was obtained from the coat formulation F3, the *in vivo* studies using human volunteers was ultimate requirement to establish their credibility. The pharmacokinetic parameter and mean plasma concentration (Table.2, Fig.3) showed that the drug was released only after 5 h indicating that the coat formulation (F1, F2, F3) has a capability of preventing the drug release in the stomach and small intestine. It was also indicated that the AUC of the formulation F3 was greater 338.53±0.89 µg hrs / ml) when compared to other formulations. *In vitro* drug release studies and *in vivo* studies using the formulation F1, F2 & F3 clearly indicated that the locust bean gum as a coat material applied over core tablet was capable of protecting the drug from being released in the physiological environment of stomach, small intestine and susceptible to colonic bacterial enzymatic action with resultant drug release in the colon. Thus, the study clearly indicated that the locust bean gum was a potential colon specific drug delivery carrier.

Table 1. Composition and thickness of Locust bean gum used to cover Mesalazine core tablets:

Formulation	Coat Weight (mg)	Composition (mg)			
		LBG	MCC	Mgs	Talc
F1	600	550	45	2	3
F2	500	450	45	2	3
F3	400	350	45	2	3
F4	300	250	45	2	3

LBG: Locust Bean Gum
MCC: Microcrystaline cellulose
Mgs: Magnesium Stearate

Table 2.:Pharmacokinetic parameter of the Mesalazine compression coated tablet obtained from the in vivo studies carried out using a 9X3 complete cross over study in healthy human volunteers.

Parameters	F1	F2	F3
C_{max} (µg\ ml)	8.64±0.038	15.92±0.11	27.51±0.03
T_{max} (h)	10±0.013	10±0.063	10±0.023
Kel (h)	0.03012±0.43	0.0513±0.11	0.0353±0.012
Bio–half life (h)	23±0.072	13±0.032	19±0.072
AUC0-t*(µg h\ ml)	147.985±0.5	249.57±0.17	480.075±0.069
AUC0-∞(µg h\ ml)	153.39±0.67	249.57±0.45	338.53±0.35
AUMC0-t*(µg h2\ ml)	2605.735±0.32	122.22±0.02	8276.85±0.49
AUMC0-t*(µg h2\ ml)	9847.49±0.01	4240.47±0.095	20084.33±0.01
MRT (h)	64.19±0.24	50.49±0.069	59.32±0.28

Each value represents mean ± S.D.

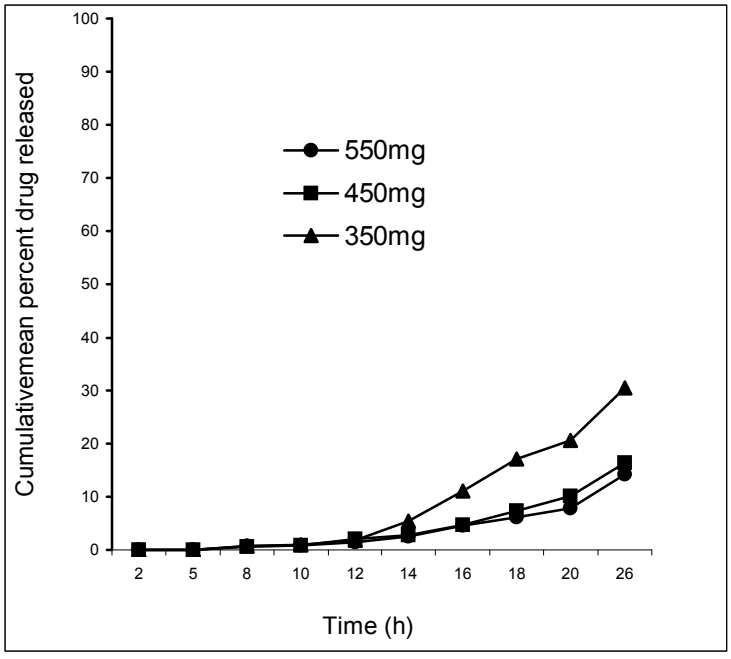

Fig.1. Cumulative mean (± S.D) percent drug released from Mesalazine tablets (n = 3) compression coated with different quantities of coating material containing 550, 450 and 350 mg of locust bean gum in 0.1 N HCl (2h), pH 7.4 buffer (3h) and pH 6.8 PBS (21h).

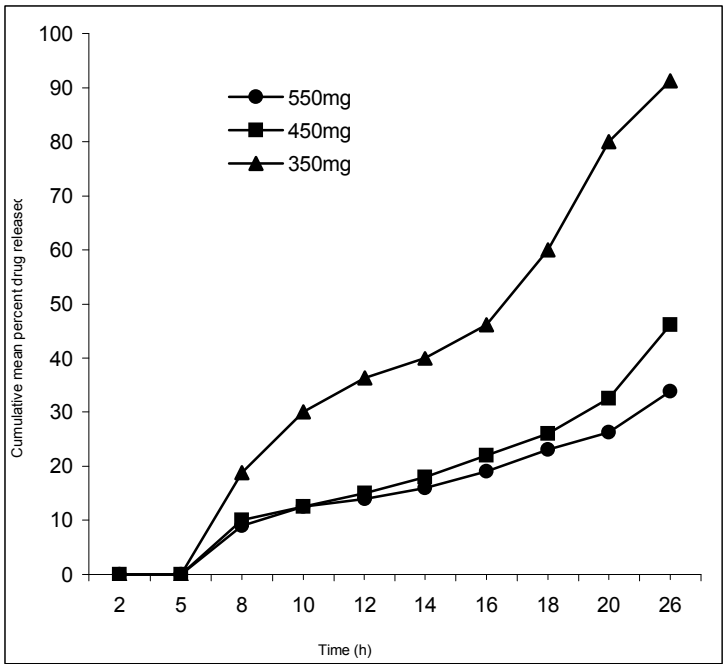

Fig. 2. Cumulative mean (± S.D) percent drug released from Mesalazine tablets (n = 3) compression coated with different quantities of coating material containing 550, 450 and 350 mg of locust bean gum in 0.1 N HCl (2h), pH 7.4 buffer (3h) and pH 6.8 PBS containing 4% w/v of rat caecal contents (21h).

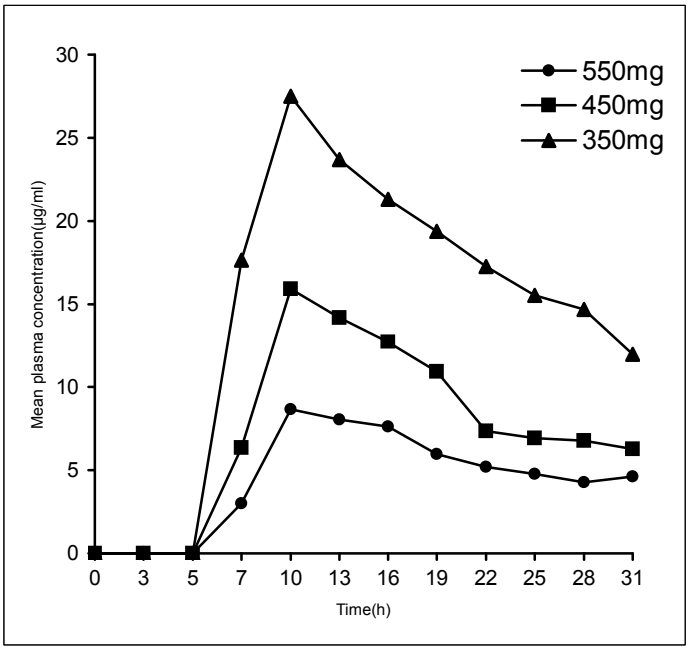

Fig. 3. Mean plasma (±S.D) concentration from Mesalazine tablets (n = 3) compression coated with different quantities of coating material containing 550, 450 and 350 mg of locust bean gum in healthy human volunteers.

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